

# Efficacy of an Herbal Extract on the Microbiological Quality of Broiler Carcasses During a Simulated Chill<sup>1</sup>

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**ABSTRACT** Protecta II, an herbal extract on an NaCl carrier, was evaluated in a 30-min, 1 C simulated chill for its effectiveness of lowering microbial counts on broiler carcasses. Eighteen broiler carcasses were obtained from a local processing plant after final wash but before chill, placed into an insulated container, and transported to the research facility for treatment. Six plant run controls (PRC) were immediately bagged on return to the pilot plant, and a whole-carcass rinse was performed. The remaining carcasses were subjected to a 30-min chill (1 C) in tap water or a 2% solution of Protecta II, (n = 6 per treatment). After treatment, carcasses were rinsed with tap water and subjected to the whole-carcass rinse procedure. All rinse diluents were microbiologically analyzed for total aerobes, coliforms, generic *Escherichia coli*, and

*Campylobacter*. Six replications were analyzed on 6 different d for a total 36 carcasses per treatment and 36 PRC. The PRC carcasses had 3.7, 2.5, 2.1, and 2.0 log<sub>10</sub> cfu/mL for total aerobes, coliforms, generic *E. coli*, and *Campylobacter*. Water treatment significantly reduced counts (2.6, 1.4, 0.7, and 0.9 log<sub>10</sub> cfu/mL, respectively) when compared with the PRC. Protecta II treatment significantly reduced counts (*P* < 0.01) even further to counts of 0.06, 0.04, 0.01, and 0.00 log<sub>10</sub> cfu/mL for total aerobes, coliforms, *Campylobacter*, and *E. coli*, respectively. Detectable levels of the monitored organisms were 1 cell/mL (log<sub>10</sub> 0) for the *E. coli*, coliforms, and total counts and 10 cells/mL (log<sub>10</sub> 1) for the *Campylobacter*. Microbial counts for carcasses treated with Protecta II would be considered too low to be detected (<1 cell/mL).

(Key words: herbal bactericide, microbiology, chilling, *Campylobacter*, *Escherichia coli*)

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## INTRODUCTION

Bacterial contamination of raw processed poultry continues to be of concern not only to consumers but also to regulatory and health officials. For the past 40 yr, scientists have been working on suitable and acceptable decontamination chemicals or processes to reduce or eliminate spoilage organisms and human enteropathogens from raw processed meat and poultry products. Addition of chlorine, hydrogen peroxide, and numerous antibiotics to processing water has reduced numbers of microorganisms in the water, but significant reductions of spoilage bacteria or human pathogens have not been noted on carcasses due to the protection afforded by the carcass itself (Thatcher and Loit, 1961; Cox et al., 1974). Lillard et al. (1987) used acetic acid in scalding water and demonstrated almost 100% reduction in microorganisms in the water but no reduction in microbial load on the carcass

itself. Treatments of the carcasses in the processing water with other chemicals, such as butylated hydroxyanisole, citric acid, lactic acid, sorbic acid, and succinic acid, have been found to reduce numbers of organisms, but as yet, none have resulted in a commercially acceptable process (Robach and Ivey, 1978; Ikeme et al., 1982). Most organic acids alter the appearance of the carcass by either bleaching and bloating or darkening the finished carcass (Mulder et al., 1987; Izat et al., 1990; Dickens et al., 1994).

Water chilling alone reduces counts on carcasses by simply removing superficial bacteria, with the washing action of commercial chillers. This reduction in counts can be negated through cross-contamination of carcasses with some of the organisms that are washed off during the chilling process. Pietzsch and Levetzow (1974) demonstrated a 50% reduction in the incidence of salmonellae-positive carcasses during immersion chilling when the flocks were heavily contaminated, but subsequent salmonellae-free flocks were also found to be contaminated with microorganisms left in the chill water from the heavily contaminated flocks. Thomson et al. (1965) and Dickens and Cox (1992) evaluated the effects of air agitation

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**Abbreviation Key:** PRC = plant run control.

on moisture pick-up, chilling time and temperature, and microbiological quality of processed poultry. The research of Dickens and Cox (1992) demonstrated that air agitation improves microbiological quality but also increases moisture pick-up above regulatory standards. Reasons for the reductions due to air agitation are not known, but Dickens and Cox (1992) suggested that it could be the scrubbing action of the bubbles produced by air being forced through the water under pressure. Dickens and Whittemore (1994) incorporated acetic acid with the air injection, which lowered moisture uptake, and significantly reduced incidence of inoculated *Salmonella* on carcasses but still had only limited effectiveness on total aerobic and Enterobacteriaceae populations.

Recently, researchers have been looking to natural means of reducing microorganisms. The use of natural antimicrobials produced from herbs and spices lends itself to more favorable acceptance by the general public as well as countries that import products treated with antimicrobials such as chlorine. Lis-Balchin et al. (1998), demonstrated the effectiveness of essential oils derived from the steam distillation of leaves of *Pelargonium* species and cultivars on numerous organisms including *Salmonella enteritidis* and *Listeria innocua*. These oils showed a greater efficacy on the *L. innocua* than did commercial thyme oil. Alcohol extracts of angelica root, banana puree, bay, caraway seed, carrot root, marjoram pimento leaf, and thyme were applied to cooked chicken to determine antimicrobial activity against *Aeromonas hydrophilia* and *Listeria monocytogenes* (Hao et al., 1998). Clove (eugenol) and pimento extract showed a 4 log reduction in growth of these organisms after 14 d of storage when compared with control samples.

Smith-Palmer et al. (1998) found that the antimicrobial properties of 21 plant essential oils and two essences were effective in reducing counts of *S. enteritidis*, *Campylobacter jejuni*, *Escherichia coli*, *Staphylococcus aureus*, and *L. monocytogenes*. The oils of bay, cinnamon, cloves, and thyme were the most effective tested for bactericidal activity. Concentrations ranging from 0.05 to 1% were determined to be bactericidal, depending on the temperature of the treatment. These oils were most effective at 35 C and decreased in effectiveness as the temperature was lowered to 4 C.

The objective of this research was to determine the effects of an herbal extract, Protecta II,<sup>3</sup> with an NaCl carrier on the microbiological quality of processed poultry carcasses during a simulated 30-min chill (1 C).

## MATERIALS AND METHODS

### General

All carcasses for the experiments were purchased from a local processor and were removed from the evisceration

line immediately after the final wash but before chill. Carcasses were placed into insulated containers to maintain carcass temperature and were transported (<12 km) to the research facility for testing. All treatments were performed within 30 min of removal from the processing line in identical prototype paddle chillers (Dickens and Cox, 1992) containing 114 L of water.

Treatment solutions were nonchlorinated water and 2% Protecta II for 45 min at 1 C; plant run control (PRC) carcasses collected after final wash prior to chill were included to delineate a baseline for carcasses prior to chill. The chilling solutions were prepared by overfilling the chillers and adding ice to the water to equilibrate the temperature to 1 C and then lowering the water level to 114 L. The treatment solutions were prepared by adding 2.25 kg of Protecta II to one chiller and an equal amount of water on a weight basis to the other. The chillers were run for 30 min prior to placing carcasses in them to insure complete mixing of the treatment solutions. Temperature and pH measurements were made with a pH meter (model CG 837<sup>4</sup>) prior to placing the carcasses into the treatment baths.

Seventy-two carcasses (six replications of six carcasses each) were subjected to the two treatments, and 36 PRC were also examined. After treatment, carcasses were rinsed with a tap water spray to remove any residual treatment solution, allowed to drip for 30 s, placed into individual bags containing 100 mL sterile water (pH 7.6), and subjected to the low-volume, whole-carcass rinse (Cox et al., 1981) with an automated shaker sampler (Dickens et al., 1985). Carcasses were removed from the shaker and allowed to drip for 30 s before the diluent was decanted into sterile specimen cups for transport to the laboratory and preparation of dilutions and plating procedures.

### Microbiological Procedures

Serial dilutions of the rinse diluent were prepared in sterile physiological saline. Total aerobic bacterial populations were enumerated on plate count agar.<sup>5</sup> One-tenth milliliter from a serial dilution of the rinse diluent was plated in duplicate on the surface of the agar, spread with a sterile bent glass rod, and incubated at 37 C for 18 to 24 h prior to counting the colony-forming units. *Campylobacter* were enumerated by plating in duplicate onto the surface of Campy-cefex agar (Stern et al., 1992). One-tenth milliliter of a serial dilution of the rinse diluent was spread on the surface of each plate with a sterile plastic- $\alpha$  bent glass rod; plates were then incubated at 42 C for 36 h in a microaerophilic environment (5% O<sub>2</sub>, 10% CO<sub>2</sub>, and balance N<sub>2</sub>). After incubation, colony-forming units characteristic of *Campylobacter* were counted. All colonies counted as *Campylobacter* from each sample were confirmed as a member of the genus by examination of cellular morphology and motility on wet mount under phase contrast microscopy. Each colony type was further characterized as a member of the species *jejuni*, *coli*, or *lari* by

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TABLE 1. pH and microbial counts for total aerobes, coliforms, *Campylobacter*, and generic *Escherichia coli* counts ( $\pm$  SD) for broiler carcasses treated with water or 2% Protecta II<sup>1,2</sup>

	pH	Total aerobes	Coliforms	<i>Campylobacter</i>	<i>E. coli</i>
		(Log <sub>10</sub> cfu/mL rinse)			
Controls	NA <sup>3</sup>	3.7 <sup>a</sup> $\pm$ 0.54	2.5 <sup>a</sup> $\pm$ 0.43	2.1 <sup>a</sup> $\pm$ 0.38	2.0 <sup>a</sup> $\pm$ 0.31
Water	7.48 <sup>a</sup> $\pm$ 0.36	2.6 <sup>b</sup> $\pm$ 0.36	1.4 <sup>b</sup> $\pm$ 0.22	0.7 <sup>b</sup> $\pm$ 0.01	0.9 <sup>b</sup> $\pm$ 0.19
Protecta II	6.31 <sup>b</sup> $\pm$ 0.27	0.06 <sup>c</sup> $\pm$ 0.01	0.04 <sup>c</sup> $\pm$ 0.01	0.01 <sup>c</sup> $\pm$ 0	0 <sup>c</sup> $\pm$ 0.0

<sup>a-c</sup>Numbers in columns with different superscripts are significantly different ( $P > 0.01$ ).

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<sup>2</sup>Total number of carcasses was 108 (six replications, six carcasses per replicate; three treatments).

<sup>3</sup>NA = not applicable.

a positive reaction with a latex agglutination test kit.<sup>6</sup> Coliform and generic *E. coli* counts were made by plating 1 mL of the serial dilution from the rinse diluent onto duplicate *E. coli* Petrifilm™ plates.<sup>7</sup> Petrifilm™ plates were incubated at 37 C for 18 to 24 h, and colony types characteristic of coliform and *E. coli* were counted.

### Statistical Analysis

General linear model (GLM), least squares means, and Tukey's studentized range test of SAS® software (SAS Institute, 1987) were used to analyze all microbiological data with treatment as the main effect and treatment by replication as the error term.

## RESULTS AND DISCUSSION

Analysis of results from the experiments demonstrated that chilling with water alone significantly reduced the microbial loads on the treated carcasses (Table 1). Log<sub>10</sub> counts per milliliter of rinse diluent were lowered by 1.1, 1.1, 1.4, and 1.1 for total aerobes, coliforms, *Campylobacter*, and generic *E. coli*, respectively. The water in which these carcasses were chilled was tap water with only minute amounts of chlorine present, below 3 ppm (unable to read free chlorine with existing equipment). Commercially processed broilers are chilled in water with 20 to 50 ppm added chlorine. Therefore, in standard commercial practices, the reduction in the microbial counts during chilling would be expected to be even greater than in the present study. Due to the logistics of ensuring all carcasses in each replication were from the same flock, chilled PRC were not evaluated.

Addition of the 2% solution of herbal extract, Protecta II, caused further reductions in the log<sub>10</sub> counts on all carcasses. These reductions were significant, with all counts being below the actual detection limit. Counts for the monitored organisms were all less than two organisms per milliliter of rinse diluent, with no *E. coli* detected on any of the treated carcasses. There was no visual color or physical change to the skin of treated carcasses, as has been observed with the use of organic acids in chiller

water (Dickens et al., 1994, Juven et al., 1974). The 2% solution of herbal extract lowers the pH of the standard tap water chilling medium by just over 1 pH unit (Table 1). No neutralization of the rinse medium was required because the pH of the herbal extract chilling solution was 6.3. The rinse procedure used in this experiment was found to be adequate to remove any residual bactericide from the carcass after treatment. This result was validated by spiking the rinse diluent with the rinse from the PRC or by adding a marker organism and plating as the standard rinse diluents were plated. All of the spiked plates showed extensive growth.

Herbs, by definition, are flowering plants whose stem above ground does not become woody and persistent. Most herbs contain various chemicals as part of their intercellular composition, and these chemicals have a demonstrated ability to help animals stay healthy when included as part of the animal diet. Additionally, these plants have developed the ability to produce chemicals over time that allows the plants to protect themselves from insects, fungi, bacteria, and viruses. When animals ingest these plants, chemicals or phytochemical extracts may give the animal some of the same protection afforded the plant. For example, the American Indian chewed the leaves and bark of willow trees to relieve aches and pains (Vandell, 1999). These leaves and bark contain the chemical salicin, which is chemically similar to the active ingredient in aspirin.

The herbal extracts in Protecta II are bacterial inhibitors that kill bacteria and reduce or inhibit surface bacterial growth. Longer contact between the product and the carcass improves the product's bactericidal effects (F. Nau, 1997, Gruenau Corp., Postfach 1063, Illertissen, Germany D-89251, personal communication). The synergistic effects of polyphenols produced from the extracts and the salt carrier are considered the basis for the bacterial action of the compound. Protecta II herbal treatment, a GRAS (generally recognized as safe) bactericide, has been cleared by the Food and Drug Administration as a processing aid and requires no additional labeling information. Preliminary work with the product demonstrated a decrease in effectiveness as the temperature of the product increased above 5 C, with best results noted between 1 and 4 C. Because this product is proprietary, no further information is available on the actual chemical composition or the basic bactericidal action. However, work is

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continuing on developing the optimum parameters for bactericidal and economic bases to make the product a feasible alternative to chlorine for poultry processing chill water.

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